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Isolation of Lamellar Bodies from Neonatal Mouse Epidermis by Selective Sequential Filtration

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Abstract. *Isolation of epidermal lamellar bodies has presented a challenge because pressures required to homogenize keratinocytes can destroy these organelles and because the lamellar body readily releases its contents during prolonged isolation procedures. In an attempt to isolate lamellar bodies, sheets of intact stratum corneum and stratum granulosum were obtained from neonatal mice with highly purified staphylococcal epidermolytic toxin, disrupted, and passed through a series of filters. The final filtrate was rich in intact lamellar bodies and contained variable amounts of ribosomes and other vesicular structures. Availability of a highly purified lamellar body preparation from postnatal epidermis should help to clarify the role of this organelle in epidermal function. The technique of selective, sequential filtration represents a new approach to cell fractionation that may have wide applications in cell biology and biochemistry.*

Orthokeratinizing epithelia contain a distinctive ovoid organelle, the lamellar body (membane-coating granule, Odland body, keratinosome) in the upper spinous and granular layers (Fig. 1) (*1*). Coincident with cornification, these organelles secrete their distinctive disclike contents into the intercellular spaces (*2*). Because ultrastructural and cytochemical studies have shown that lipids (*3*), sugars (*4*), and hydrolytic enzymes (*5*) are present in these organelles, they have been considered the primary source of materials for skin barrier function (presumably through their lipids) (*3*, *6*) and for cohesion and desquamation (pre-

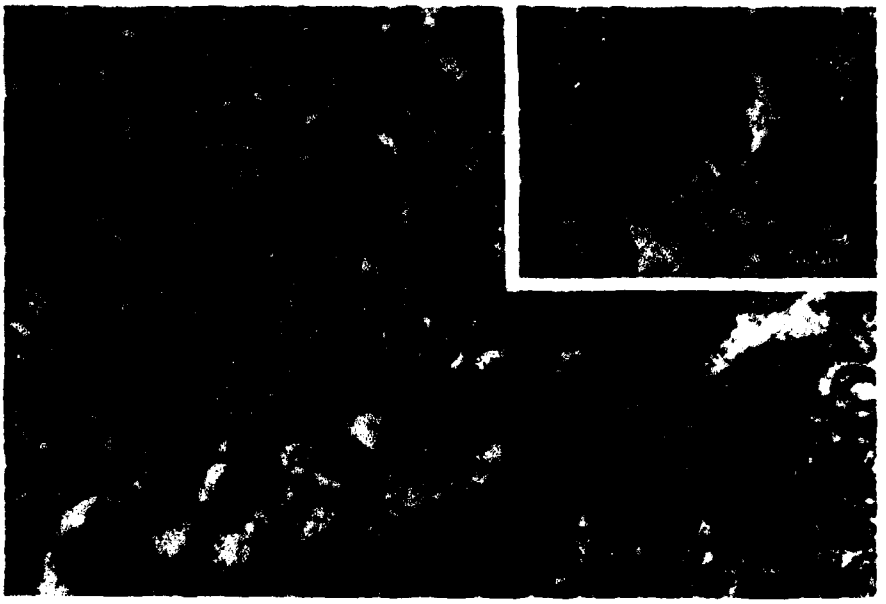

Fig. 1. Thin section through the stratum granulosum (*SG*) of neonatal mouse whole epidermis. The cytoplasm contains aggregates of intermediate filaments, keratohyalin granules, free ribosomes, occasional mitochondria, and abundant lamellar bodies (*LB*). The latter are ovoid, membrane-delineated organelles with a mean diameter of 0.10 to 0.20 μm. In cross sections they display stacks of parallel discs, but in grazing sections the contents look amorphous (inset). The granular cell is notably devoid of endocytic vesicles and contains only sparse rough and smooth endoplasmic reticulum.

sumably through their sugars and hydrolytic enzymes) (*5*). Attempts to isolate lamellar bodies from postnatal keratinizing epithelia have been frustrated by the resistance of human and mouse epidermal granular cells to homogenization (the pressures required for rupture often shear the lamellar body's limiting membrane) and by the rapidity with which these organelles release their contents during tissue preparation. Although a method for the partial purification of lamellar bodies from fetal rat epidermis was recently reported (*7*), it apparently is not applicable to skin ex utero because of the greater pressures required to rupture more differentiated cells, and it is in the postnatal state that the skin presumably elaborates those substances required for barrier function and desquamation.

To circumvent the dual problems of homogenization and prolonged isolation procedures, we developed a novel isolation scheme that combines controlled homogenization by a cell disruptor (*8*) with purification through a series of filters of decreasing pore size. This technique exploits the small size of the organelles (0.15 to 0.20 μm), effectively excluding most other subcellular structures. The entire process takes less than 30 minutes, unlike differential or isopyknic techniques, which require much longer periods.

Intact sheets of stratum corneum and stratum granulosum were obtained by intradermal injection of 20 μg of a highly purified fraction of staphylococcal epidermolytic toxin (*9*). The sheets were suspended in ice-cold DME culture medium (free of Ca^{2+} and glucose) with 20 m*M* hepes and 10 m*M* EDTA (*p*H 6.5), rinsed, homogenized in a loose-fitting, ground-glass homogenizer, and filtered through gauze. The filtrate was further sheared in a Stansted cell disrupter at 5000 lb/in^2. The disrupter consisted of an air-driven, high-pressure liquid pump that forced the cell-containing liquid through a back pressure valve. The degree of cell breakage is controlled by the force of a stainless steel ball against a narrow orifice. A water-jacketed reservoir was used to keep all fluid ice-cold during disruption. After 10 minutes of centrifugation at 700*g*, the supernatant was passed through a Millipore filter with a pore size of 8 μm and then a series of 25-mm-diameter Nuclepore filters with pore sizes of 8.0, 3.0, 1.0, 0.8, 0.6, 0.4, and 0.2 μm. Approximately 30 ml of homogenate, taken up in a syringe, was passed through each filter until minimum resistance was felt before advancing to the next smaller pore size. Portions of about 5 ml were used for each 0.2-μm filter to avoid excessive clogging. Filtrates were centrifuged at 20,000*g* and frozen or fixed as required for biochemical analysis or electron microscopy.

As shown in Fig. 2, the final filtrate was rich in intact lamellar bodies. More important, most of the organelles had an undamaged limiting membrane and disc-like or amorphous contents that were comparable to those found in whole epidermis. As in whole epidermis, lamellar contents were not visible in some cases because of tangential sectioning resulting from the short cross-sectional diameter of these organelles (0.15 μm, compared to the 0.6- to 0.8-μm thickness of ultrathin tissue sections) (*10*) and their high radius of curvature. In addition to lamellar bodies, the final filtrate contained abundant ribosomes, some granules with

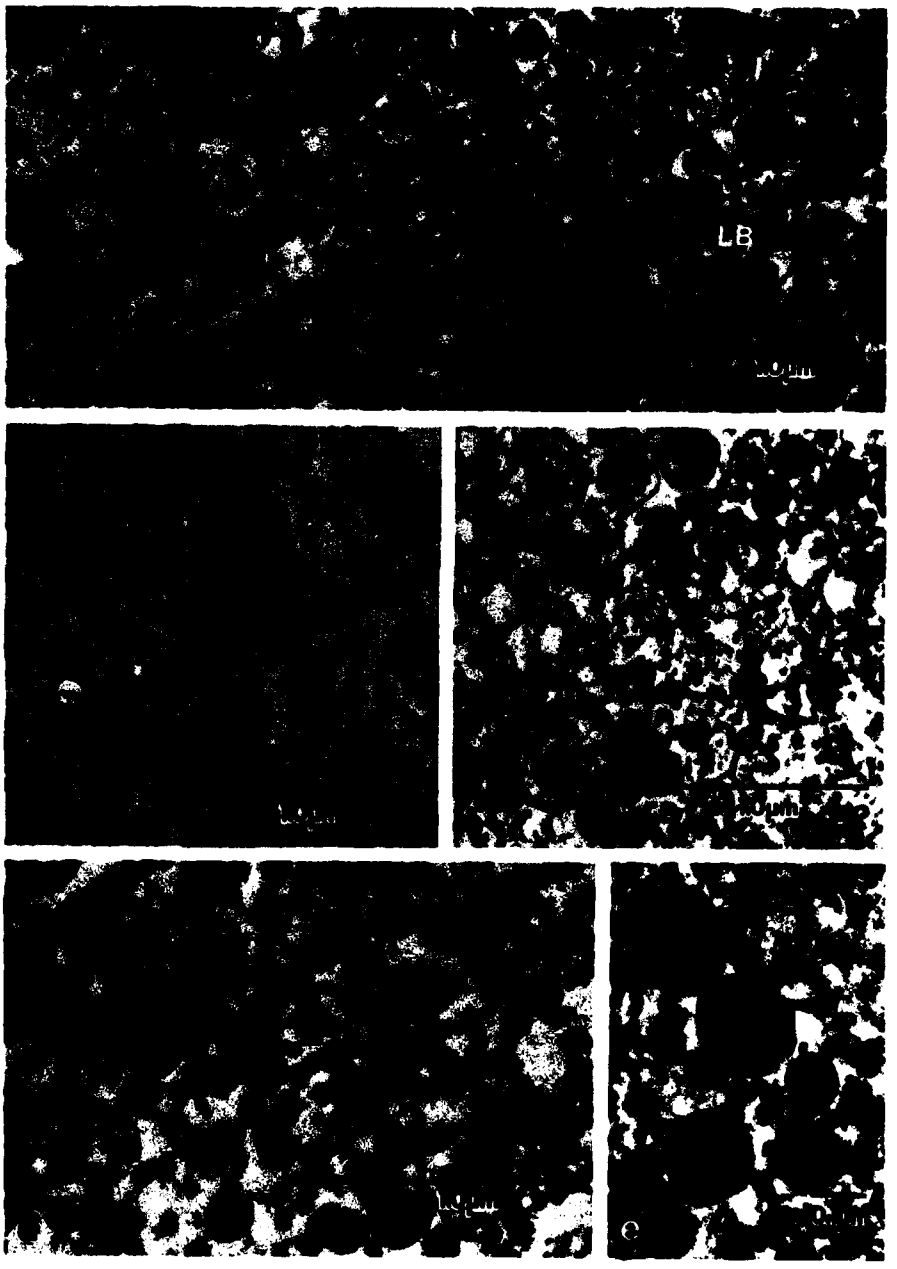

Fig. 2. Selected thin sections of representative filtrates obtained during homogenization and sequential filtration. (a) Crude homogenate, containing the full panoply of subcellular structures present in intact epidermis, including bundles of keratin filaments, keratohyalin granules, various membrane structures, disrupted cornified cell envelopes, and many lamellar bodies. The filtrates obtained with pore sizes of 1, 0.4, and 0.2 μm are shown in (b), (c), and (d), respectively. Although each contains intact lamellar bodies, only the filtrates represented in (c) and (d) are rich in lamellar bodies. In the filtrate obtained with a pore size of 0.2 μm, lamellar bodies constitute 40 to 50 percent of the cellular structures. Lamellar bodies in the final filtrate (e) have the same internal structure as those in intact skin (inset in Fig. 1).

the characteristic crystalline shape of glycogen, and lesser amounts of smooth membrane vesicles.

Two to four grams (wet weight) of starting material (10 to 20 litters of neonatal mice) yielded a final pellet of about 5 mg (wet weight)—a quantity sufficient for further biochemical characterization. Since neonatal skin possesses the full complement of organelles found in the adult, this technique should be directly applicable to adult epidermis.

This preparation should make it possible to ascertain the precise functions of the epidermal lamellar body (*5*, *6*). Although a comprehensive survey of the organelle's contents is not yet completed, preliminary examination indicates that it has a lipid-to-protein ratio of about 40 to 60, that it has a higher ratio of glycosphingolipids to ceramides (1.29) than crude fractions (0.69), and that it is not richer than other membrane fractions in lysosomal enzymes. Finally, we believe that the method described here will have many applications in cell biology and biochemistry, particularly in situations where a particularly fragile or labile structure must be preserved.

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